Validation of a process specific ELISA for the monitoring of HCP

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Introduction

The development of biological therapeutic products has been booming for years. A critical aspect for product manufacturing is the release of impurities such as host cell proteins (HCP), which affect drug efficacy and might compromise patient safety. HCP are proteins and polypeptides coming from the host organism, which may contaminate drug substance (DS) and that can be detected and quantified by sandwich ELISA using polyclonal sera raised against HCP.

To quantify HCP, a generic HCP kit is usually used during each phase of the purification bioprocess, but from Phase III, it is strongly recommended to develop a process specific HCP ELISA kit. Another scenario may require to go directly with a process specific kit : when the general kit is no longer well adapted, because of a deficient coverage or because of the cross reactivity against the DS.

In this context, the regulatory authorities require an adequate coverage from polyclonal antibodies (pAbs) on HCP population and to maintain HCP level in drug samples within 1-100 ng/mL. The aim of this poster was to present coverage and cross reactivity data, and to demonstrate validation of a process specific anti-HCP ELISA.

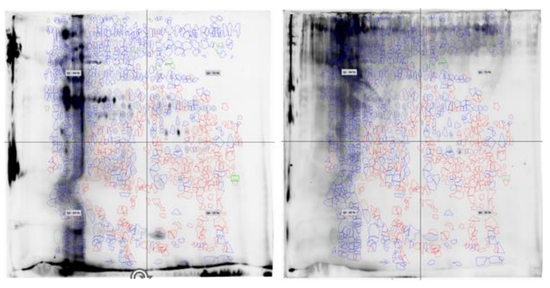
Coverage

The coverage evaluation is as estimation of the percentage of HCP that can be detected by anti-HCP antibodies.

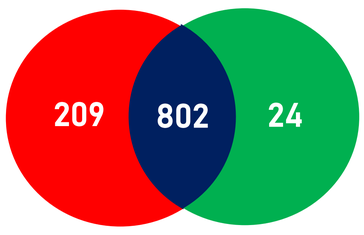
1. Sample preparation by 2D Clean-up and protein quantification
2. Cyanine 3 labelling of HCP
3. Study of the impact of the cyanine 3 labelling by 2D electrophoresis
4. Coverage analysis 2D-DIBE : Cy3 labeled HCP spots versus detected spots using anti-HCP antibodies labeled with Cy5

Used products : purified rabbit anti-process specific HCP pAbs (50 µg/mL)

Results: **Coverage rate = 80%**



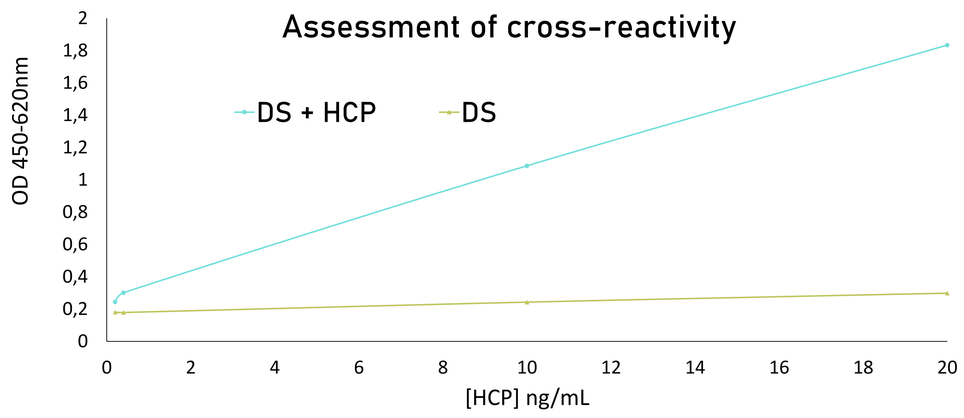
Cya3 detection Cya5 detection



Total HCP	pI < 5,8	pI > 5,8
MW > 32k Da	Q1 – 94 %	Q2 – 82 %
MW < 32k Da	Q3 – 69 %	Q4 – 37 %

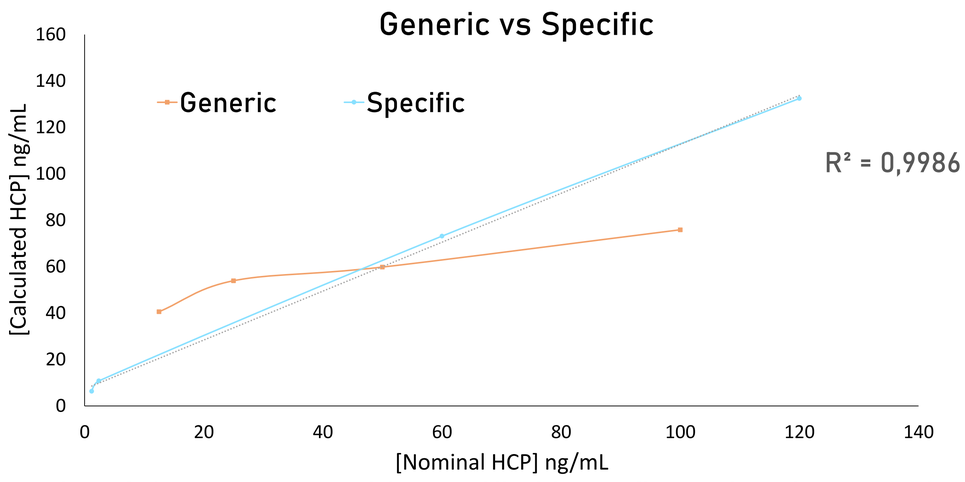
Cross-reactivity

To detect potential cross-reactivity between DS and anti-HCP antibodies, DS is serially diluted and tested as a sample with and without HCP spike.



The signal from DS alone is close to 0.2 and is lower than the signal obtained when HCP are spiked into the DS.

To compare comportment in generic kit



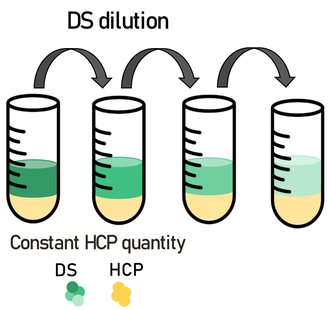
Linearity of the response with specific kit confirms the absence of cross-reactivity

Linearity

Minimal Required Dilution (MRD) :

MRD should be established by HCP spiking in sample dilution series. HCP at 5 ng/mL are spiked in 2-fold serially diluted DS.

Minimize matrix interference as much as possible by assessing the potential matrix effect between standard buffer and DS samples.



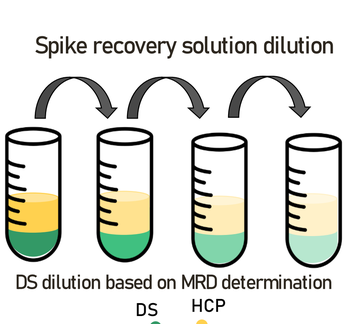
DS Dilution	[DS] mg/mL	[Calculated HCP] ng/mL	% recovery (70-130%)
1/2	1	2,36	47,17
1/4	0,5	3,36	67,19
1/8	**0,25**	**4,13**	82,57
1/16	0,125	4,57	91,34
1/32	0,0625	4,55	90,95
1/64	0,03125	4,47	89,39
1/128	0,015625	4,47	89,31
1/256	0,0078125	4,43	88,62

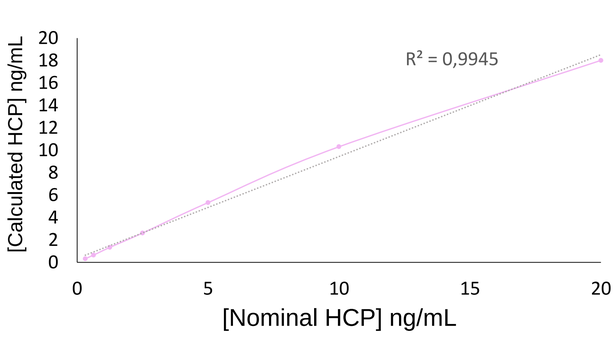
MRD = 1/8 corresponding to DS at 0,25 mg/mL

Sample linearity :

To confirm lack of cross-reactivity of the anti HCP antibodies with the product itself, HCP accuracy is evaluated in the DS and accurate spike recovery demonstrated over the range of the assay.

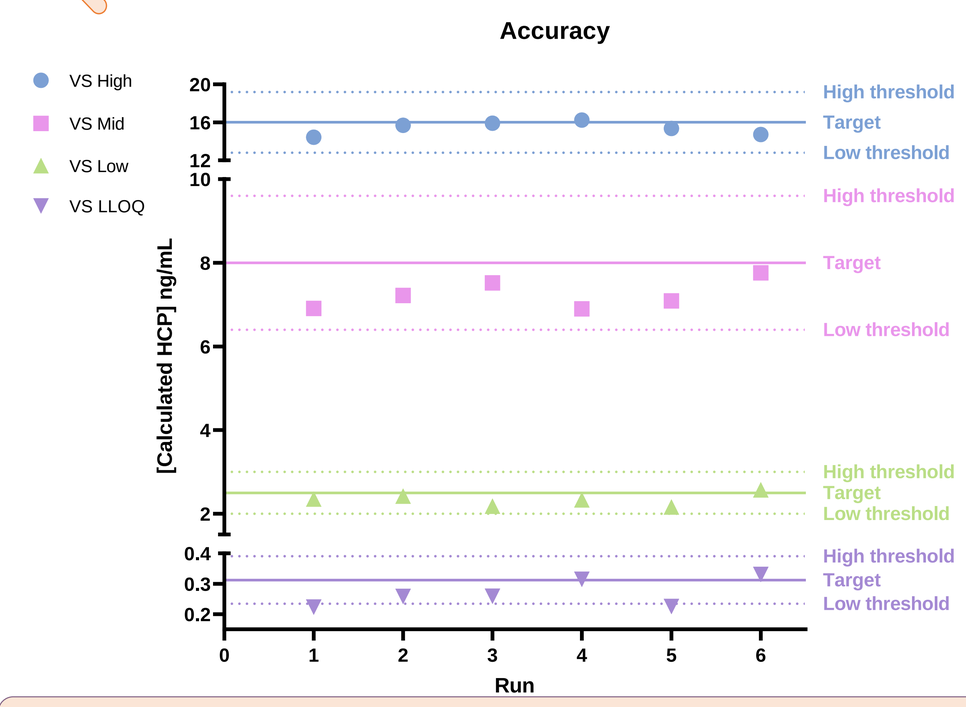
Check the ability to measure analyte concentrations along HCP range using DS MRD.



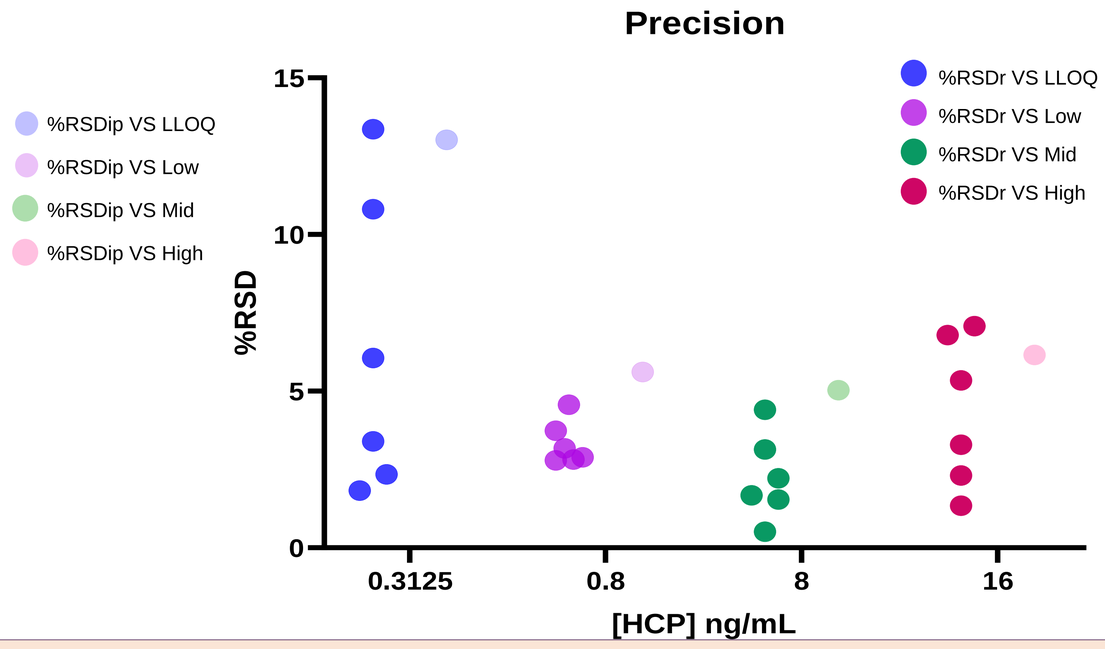


Accuracy Precision

Accuracy = the closeness of test results obtained by that procedure to the true value.



Precision = the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the same conditions.



Conclusion

After demonstration of adequate coverage from polyclonal antibodies on HCP population by 80%, which is considerate as a high rate, the process-specific ELISA test was successfully developed and validated. The test did not show any cross-reactivity, leading to conform linearity of the DS over HCP range with no matrix effect from DS diluted from 1/8. Accuracy and precision demonstrated the sensitivity of detection and accurate quantification over HCP range.

Comparing performance with a generic kit, necessity to develop process specific test is well demonstrated.